PB81-168726

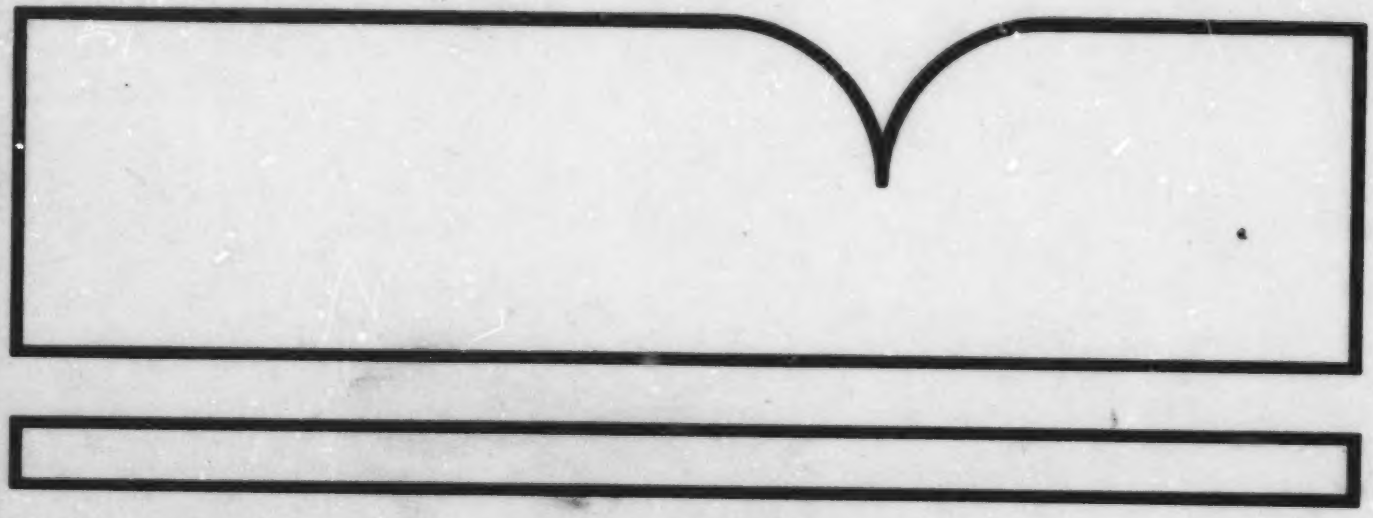
Interrelationship of Bacterial Counts with Other Finished Water Quality Parameters within Distribution Systems

Salem and Beverly Water Supply Board
Beverly, MA

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

Feb 81



U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO. PB81 168726
4. TITLE AND SUBTITLE Interrelationship of Bacterial Counts with other Finished Water Quality Parameters Within Distribution Systems		5. REPORT DATE February 1981 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) J. Kevin Reilly and Joyce S. Kippin		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Salem and Beverly Water Supply Board Beverly, MA 01915		10. PROGRAM ELEMENT NO. BNCIA 11. CONTRACT/GRANT NO. R-804724
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory - Cin., OH Office of Research and Development U.S. Environmental Protectin Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED Final: 11/1/76-1/31/79 14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES
Project Officer: Raymond H. Taylor (513) 684-7204

16. ABSTRACT

The objective of this research program was to obtain realistic information concerning the interrelationships between temperature, chlorine, turbidity, coliforms, and Standard Plate Count (SPC) densities present in finished water after treatment and distribution. Bacterial identifications were performed to determine types and densities of isolates from the SPC and coliform tests.

The frequency of coliform isolation was independent of free chlorine, turbidity, and temperature. SPC were not contingent upon low level turbidity and varied with respect to free chlorine residual and temperature. SPC exhibited no interrelationship with coliform counts (MF) when the SPC was less than 50 organisms/mL. A slight inverse relationship was noted between free chlorine residual and turbidity. Of the physical and chemical parameters measured, free chlorine residual had the greatest influence on the microbial population.

Encapsulated Klebsiella pneumoniae, Enterobacter agglomerans, Enterobacter aerogenes and Enterobacter cloacae exhibited the ability to survive a free chlorine residual of 0.2 mg/L or more. The diversity of organisms identified by the SPC method strongly suggests the phenomenon of an established microbial ecosystem with the distribution networks.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Chlorination Potable Water Coliform Bacteria Water Distribution	Bacterial Identification Standard Plate Count	13 B

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	53
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

EPA 600/2-81-035
March 1981

PB81-168726

INTERRELATIONSHIP OF BACTERIAL COUNTS WITH OTHER FINISHED WATER QUALITY PARAMETERS WITHIN DISTRIBUTION SYSTEMS

BY

J. Kevin Reilly
Joyce S. Kippin
Salem and Beverly Water Supply Board
Beverly, Massachusetts 01915

6-1/2"
9-1/8"

Grant No. R804724

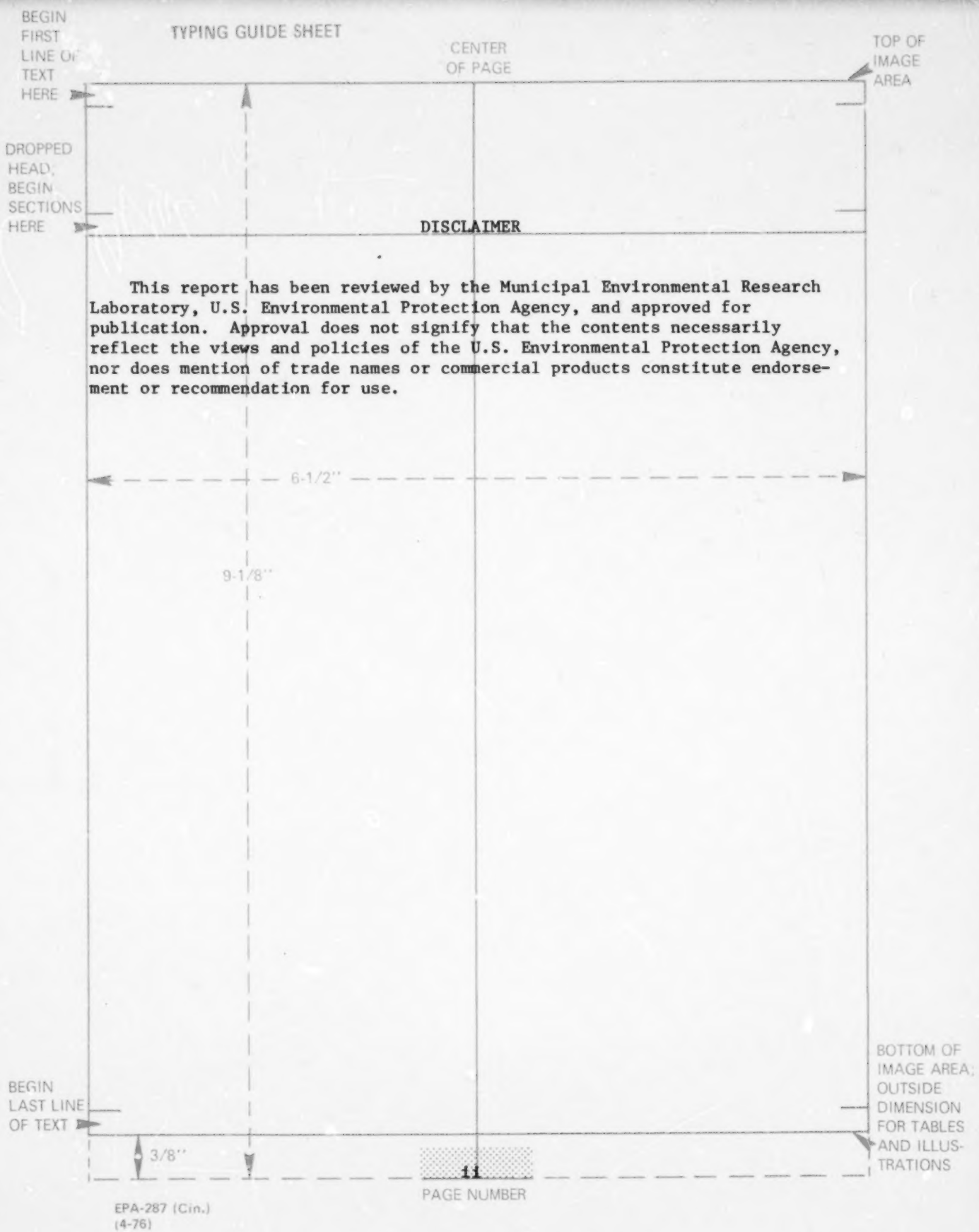
Project Officer

Raymond H. Taylor
Drinking Water Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.) (4-76)


BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
DISCLAIMER
This report has been reviewed by the Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.
6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
ii
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and mangement of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the prservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This report evaluates the interrelationships between temperature, chlorine, turbidity, coliforms, and Standard Plate Count densities present in finished water. Levels of these parameters, interpretation of data, and biochemical characteristics of these bacteria are presented for samples from both the Salem, Massachusetts and Beverly, Massachusetts, distribution systems.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-267 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD. BEGIN SECTIONS HERE

PREFACE

Recently, increased attention has been placed upon water quality and the monitoring procedures that ultimately determine the quality of water delivered to the consumer. Questions have been raised about bacterial, chemical, and physical standards used in the water quality monitoring and their interrelationships in the final product reaching the tap. These standards and some of their relationships have been studied and documented in the laboratory and in distribution systems. However, previous studies have tended to focus on those systems that do not provide full treatment to their water supply and have not generally monitored seasonal fluctuations of the above parameters. Water supplies providing full treatment (chlorination, rapid mixing, flocculation, sedimentation, filtration) have tended to be ignored in previous studies, presumably since they were assumed to be supplying good and safe water to their consumers. "Good" quality has been defined as that which can meet or be better than those standards set for water supplies by the appropriate regulatory agencies. The presumably good quality water supplies that have been studied were examined only with respect to one, two or three parameters within the confines of narrow goals set as their end product. Hence, it was determined that two separate and distinct water distribution systems, both having a common history and having met the U.S. Public Health Service standards for common carrier since 1935, should be comprehensively studied to determine if certain aspects of the drinking water standards adequately control the quality of finished water in the distribution system.

Good quality water leaving the treatment facility has long been known to undergo deterioration within the distribution system, but the extent of chemical, physical, and biological degradation before the water reaches the consumer has not yet been fully studied. Therefore, the objective of this research was to determine if monitoring of basic water quality parameters adequately characterizes the microbial quality of water as it travels through the distribution system.

As with any program of study there were unexpected occurrences that altered the scope of the above objectives. Many of our early assumptions regarding distribution systems and the standards used to measure water quality were changed in the light of the results obtained from the research program. Consequently, the scope of the research project was greatly enlarged by the results obtained during the study.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA. OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

ABSTRACT

The objective of this research program was to obtain realistic information concerning the interrelationships between temperature, chlorine, turbidity, coliforms, and Standard Plate Count (SPC) densities present in finished water after treatment and distribution. Bacterial identifications were performed to determine types and densities of isolates from the SPC and coliform tests.

The frequency of coliform isolation was independent of free chlorine, turbidity, and temperature. SPC were not contingent upon low level turbidity and varied with respect to free chlorine residual and temperature. SPC exhibited no interrelationship with coliform counts (MF) when the SPC was less than 50 organisms/ml. A slight inverse relationship was noted between free chlorine residual and turbidity. Of the physical and chemical parameters measured, free chlorine residual had the greatest influence on the microbial population.

Encapsulated Klebsiella pneumoniae, Enterobacter agglomerans, Enterobacter aerogenes and Enterobacter cloacae exhibited the ability to survive a free chlorine residual of 0.2 mg/L or more. The diversity of organisms identified by the SPC method strongly suggests the phenomenon of an established microbial ecosystem with the distribution networks.

This report was submitted in fulfillment of Grant No. R 804724 by the Salem and Beverly Water Supply Board under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period November 1, 1976 to January 31, 1979.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

CONTENTS

Foreword iii
Preface iv
Abstract v
Figures viii
Tables viii
Acknowledgment ix

1. Introduction 1

2. Conclusions 3

3. Recommendations 4

4. Materials and Methods 6

General procedure 6

Sampling procedures 6

Methods of analysis 6

5. Results and Discussion 13

Program I 13

Program II 17

Program III 26

Raw and finished water parameters 28

Parameter ranges and those not analyzed 28

Confirmation procedure. 29

Beverly's reversal of flow 29

Chlorine coliform experiments 31

Microbiological identification section 31

References 39

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

FIGURES

Number		Page
1	Sampling collection and analysis	7
2	Bacterial identification technique	9

TABLES

1	Test Statement 1.	Bacterial Density Vs. Free Chlorine	14
2	Test Statement 2.	Bacterial Density Vs. Temperature	15
3	Test Statement 3.	Bacterial Density Vs. Turbidity	15
4	Test Statement 4, 5 and 6.	SPC Density Vs. Coliform Density, Turbidity Vs. Free Chlorine, Turbidity Vs. Temperature	16
5	Test Statement 1.	Effect Of Free Chlorine On Coliform Densities In Two Distribution Systems.	18
6	Test Statement 2.	Effect Of Free Chlorine on SPC Densities In Two Distribution Systems.	20
7	Test Statement 3.	Effect Of Turbidity On Coliform Densities In Two Distribution Systems.	21
8	Test Statement 4.	Effect Of Turbidity On SPC Densities In Two Distribution Systems	22
9	Test Statement 5.	Effect Of Turbidity On Free Chlorine Residuals In Two Distribution Systems.	23
10	Test Statement 6.	Effect Of SPC Densities On Coliform Densities In Two Distribution Systems.	24
11	Test Statement 4.	Effect Of Temperature On SPC And Turbidity	25
12	Coefficient Of Determination For Free Chlorine, Total Chlorine, Coliform, SPC, Turbidity And Temperature		27
13	Raw And Finished Water Parameters.		28
14	Parameter Range Of Distribution System Samples		29
15	Microbiological Identifications Of Organisms Isolated Using The Membrane Filter Procedure For Coliforms And the Standard Plate Count.		32

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

ACKNOWLEDGMENTS

The cooperation, support and contributions of Raymond H. Taylor, EPA Project Officer, and Mario J. Tricomi, laboratory consultant, are gratefully appreciated and acknowledged.

6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-78)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

SECTION 1

INTRODUCTION

The lack of information regarding problems associated with the distribution of acceptable quality water to the consumer emphasizes the need for a better understanding of water quality deterioration after complete treatment. Many papers have been written about unfiltered water supplies (1-9), dead end mains (10-15,1), pipe (16-31,12), reservoir disinfection (32-34,1) and other problems associated with marginal water (35,16) supplies within a distribution system.

This two year study has attempted to provide much needed insights into seasonal variations of water quality due to changing interrelationships among such fundamental parameters as temperature, chlorine residual, turbidity, pH, coliforms and Standard Plate Counts (SPC). In addition to the quantitative aspects of these parameters, a qualitative analysis of organisms isolated from the finished water supply was conducted.

The communities of Salem and Beverly have been supplying water to their citizens since 1792 and 1836 respectively. It was not until 1869 that both cities had a common source water, Wenham Lake. In Salem much of the 10 and 15 cm (4-6 inch) cast iron mains were laid in the 1890's, however, after the Great Fire of 1914 (1792 buildings were destroyed) 41 and 51 cm (16-20 inch) cast iron lines were installed to provide better fire fighting capability. Some of these larger mains were dead ended and most of the services remained connected to the smaller pipes laid before the fire. In 1935, both cities under the aegis of the Salem and Beverly Water Supply Board put into operation a 30 million liter (8 million gallon) filtration plant, consisting of conventional treatment and rapid sand filters to meet increasing demands (36). Both municipalities pumped from the plant's 15 million liter (4 million gallon) clearwell into their separate distribution systems. Presently, Salem has approximately 143 kilometers (89 miles) of pipe, including a great deal of the original cast iron mains from the 19th century, delivering 24 million liters (6.3 million gallons) of water a day to its consumers. The Salem system has two pressure zones and two storage reservoirs totaling 58 million liters (15.3 million gallons). The detention time of the water in the distribution system has been estimated to range from 2 to 7 days (37). Most of Beverly's 206 kilometers (128.5 miles) of cast iron pipes are more recent than Salem's network. Also, Beverly does not have any large dead end mains in its looped system. The distribution system has two small high pressure zones within itself and two 14 million liter (3.7 million gallon) reservoirs, supplying Beverly's 16 million liter (4.2 million gallon) daily consumption. Neither city has a flushing program but they do receive an anticorrosion bimetallic phosphate treatment from the filtration plant. It should be noted

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
the filtration plant was under extensive renovation and reconstruction to upgrade it to a 91 million liter (24 million gallon) facility. Consequently, only five of eight filters and two of four settling basins were in service for periods of time during the study.
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
2
PAGE NUMBER
EPA-287 (Cin.) (4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

SECTION 2

CONCLUSIONS

The distribution systems of Salem and Beverly have established microbial ecosystems within their distribution networks. The similarity of the ecosystems' bacterial isolates from the two separate and distinct distribution systems was probably related to sharing the same source water and treatment processes.

Coliform bacteria were found to be a part of the ecosystems established within the distribution systems.

The occurrence of coliforms in the distribution networks was independent of free chlorine residuals present in the water supplied by the filtration plant.

The occurrence of coliforms was independent of turbidity fluctuations of less than 2.0 NTU in both distribution systems supplied by the filtration plant.

The occurrence of coliforms within a distribution system was either very slightly dependent or independent of temperature fluctuations.

Free chlorine residuals had a slight inverse effect on the occurrence of SPC populations.

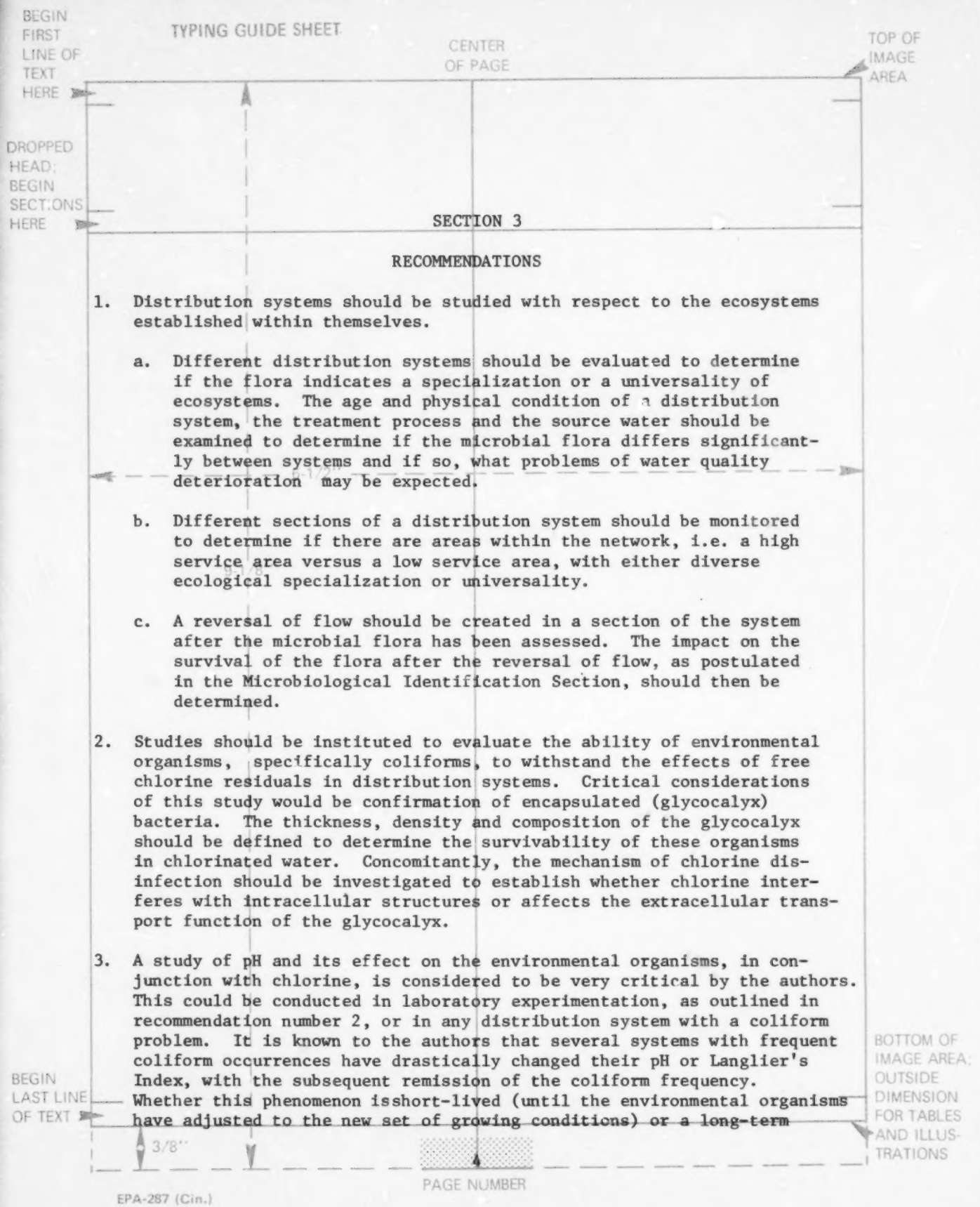
SPC populations were independent of turbidity fluctuations of less than 2.0 NTU.

SPC of 50 colonies or less did not reflect the presence or absence of coliforms.

There was a slight inverse relationship between free chlorine residual and low level turbidity, but it was not demonstrated to be statistically significant.

Of the parameters measured, free chlorine residual demonstrated the greatest effect on the microbial flora.

Analysis of all the parameters in the above conclusions proved to be difficult due to the variation of physical and chemical processes at the filtration plant and the inherent complex nature of the dynamic ecosystems within the distribution networks.



SECTION 3

RECOMMENDATIONS

1. Distribution systems should be studied with respect to the ecosystems established within themselves.

   a. Different distribution systems should be evaluated to determine if the flora indicates a specialization or a universality of ecosystems. The age and physical condition of a distribution system, the treatment process and the source water should be examined to determine if the microbial flora differs significantly between systems and if so, what problems of water quality deterioration may be expected.

   b. Different sections of a distribution system should be monitored to determine if there are areas within the network, i.e. a high service area versus a low service area, with either diverse ecological specialization or universality.

   c. A reversal of flow should be created in a section of the system after the microbial flora has been assessed. The impact on the survival of the flora after the reversal of flow, as postulated in the Microbiological Identification Section, should then be determined.

2. Studies should be instituted to evaluate the ability of environmental organisms, specifically coliforms, to withstand the effects of free chlorine residuals in distribution systems. Critical considerations of this study would be confirmation of encapsulated (glycocalyx) bacteria. The thickness, density and composition of the glycocalyx should be defined to determine the survivability of these organisms in chlorinated water. Concomitantly, the mechanism of chlorine disinfection should be investigated to establish whether chlorine interferes with intracellular structures or affects the extracellular transport function of the glycocalyx.

3. A study of pH and its effect on the environmental organisms, in conjunction with chlorine, is considered to be very critical by the authors. This could be conducted in laboratory experimentation, as outlined in recommendation number 2, or in any distribution system with a coliform problem. It is known to the authors that several systems with frequent coliform occurrences have drastically changed their pH or Langlier's Index, with the subsequent remission of the coliform frequency. Whether this phenomenon isshort-lived (until the environmental organisms have adjusted to the new set of growing conditions) or a long-term

FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.) 4-76)

effect (such as would be the case if the acidic coat were removed by the alkaline water, making the organisms susceptible to destruction by chlorine) can only be determined by further studies.

4. Additional research should be undertaken to study the SPC enumeration procedures along with improved media and new recovery methods and to profile the SPC population so as to define the health significance and impact on coliform populations by these organisms in the microbial flora.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

SECTION 4

MATERIALS AND METHODS

GENERAL PROCEDURES

All laboratory procedures, unless otherwise stated, were performed in accordance with the 14th edition of Standard Methods for the Examination of Water and Wastewater (38).

SAMPLING PROCEDURES (FIGURE 1).

Sample Collection

The sample analysis technique is outlined in Figure 1. Samples were collected weekly on Monday or Tuesday morning over a 23 month period (11/1/76) thru 9/18/78. Thirteen samples were collected each week from the Salem distribution system, 12 from the Beverly distribution system, and 2 from the filtration plant (raw and finished).

The sampling locations in both cities were faucet taps located in public buildings in the middle and ends of each distribution network to give a representative perspective of both systems. One of Salem's 13 samples was the Salem Pumping Station which is located immediately after the 15 million liter (4 million gallon) clearwell of the filtration plant. A total of 1,157 samples was collected in Salem, 1,056 samples in Beverly, and 196 samples in the filtration plant, to make a grand total of 2,409 for the 2 year study.

On Site Measurements

Temperature and chlorine measurements were performed at the sampling points. Total and free chlorine measurements were done using a Hach DPD kit. The time interval between sample collection and receipt in the laboratory was less than 3 hours.

METHODS OF ANALYSIS

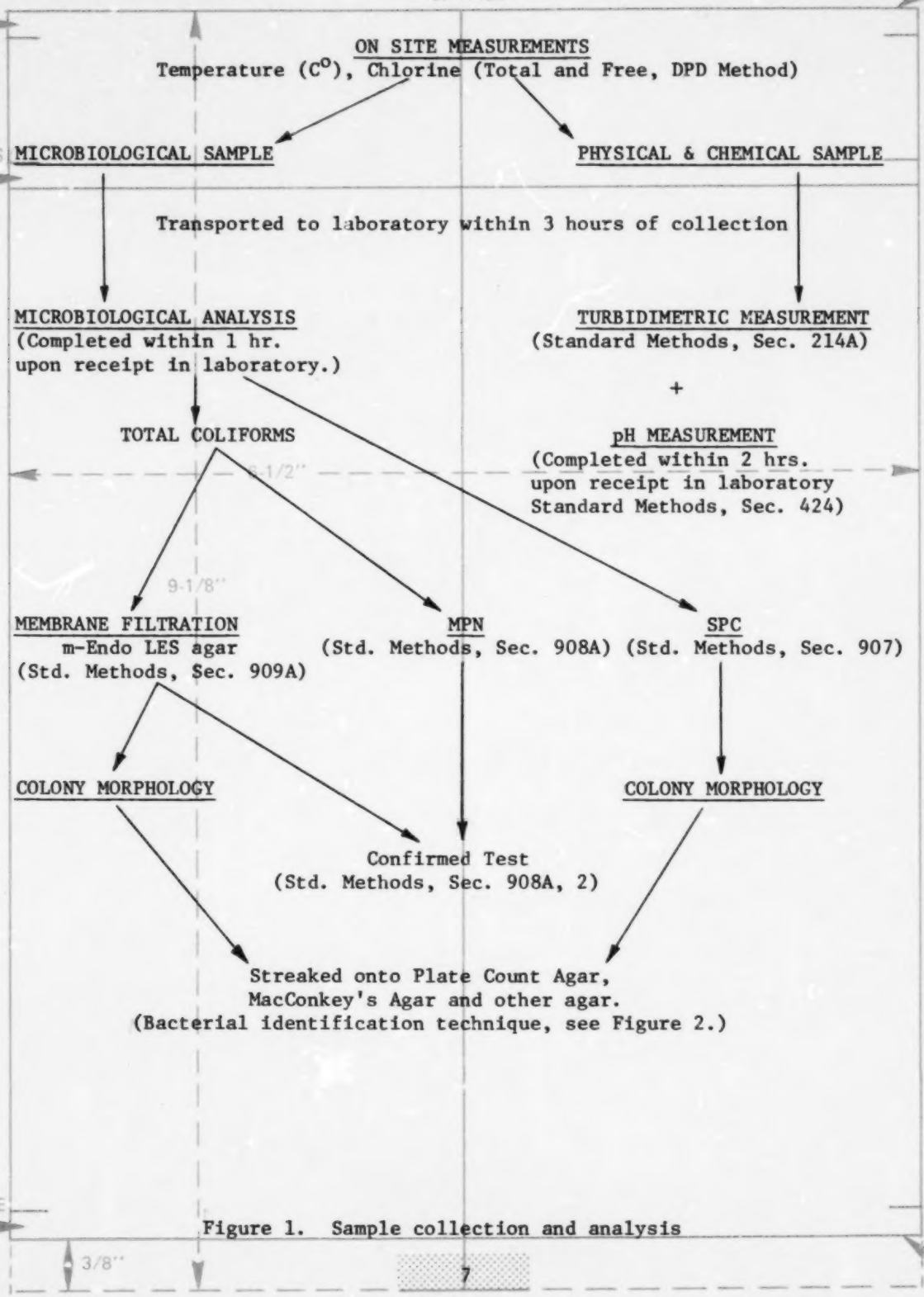
pH and Turbidimetric Measurements

pH and turbidity measurements were made within two hours of receipt in the laboratory. The turbidity readings were made using the Hach 2100A turbidimeter. The pH measurements were done electrometrically with a Radiometer PM26.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE



BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
BEGIN LAST LINE OF TEXT

Figure 1. Sample collection and analysis

3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

Microbiological Analyses

All of the microbiological analyses were performed immediately upon reception of the samples in the laboratory and completed within an hour of receipt. The SPC's were done in triplicate using Tryptone Glucose Extract Agar during the first half of the study and Plate Count Agar for the second half of the program. Plates were counted after 48 hours incubation at 35°C. The MPN test for total coliforms was done weekly on both raw and finished water samples from the filtration plant and two randomly selected samples from each of the two cities. The presumptive media used in the MPN tubes was Lauryl Tryptose Broth (LTB). All presumptive positive tubes were carried through to the Confirmed Test procedure. The membrane filter total coliform test was run on all samples, using m-Endo Agar LES. Typical coliform colonies were confirmed in LTB, then Brilliant Green Bile for gas production within 48 hours at 35°C.

Bacterial identifications were done using Bergey's Manual of Determinative Bacteriology in conjunction with the API 20E gram negative rod identification system. Roche Diagnostic's Enterotube was also used to identify some organisms from the membrane filters. Organisms that grew on the membrane filters and pour plates were randomly selected and streaked onto both Plate Count Agar and MacConkey Agar. Those organisms determined to be Gram negative rods were further classified and speciated using the biochemical tests of the commercial API 20E system. If the identified organism was other than a gram negative rod, Bergey's Manual was consulted for the necessary criteria to identify the organism. These procedures are displayed in **Figure** 2.

Data Analysis

The massive amount of data gathered was placed on cards and analyzed with the aid of the UNIVAC 1108 computer. Three types of data analysis were used to insure the validity of the methods in analyzing the data and to confirm the conclusions.

In the first method, Program 1, the data was analyzed using 9 statements. The data for both distribution systems were combined to give one resulting percentage for each statement. A typical statement was, "SPC decreased when free chlorine residual increased". The results were expressed as a percentage when both conditions of the statement were met. The complete set of statements used in categorizing and analyzing the data are as follows:

1. a. Coliform density decreased or remained at 0 when free chlorine residual increased.

   b. SPC decreased when free chlorine residual increased.

2. a. Coliform density increased when the water temperature increased.

   b. SPC increased when the water temperature increased.

3. a. Coliform density increased when the turbidity increased.

BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
BEGIN LAST LINE OF TEXT
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

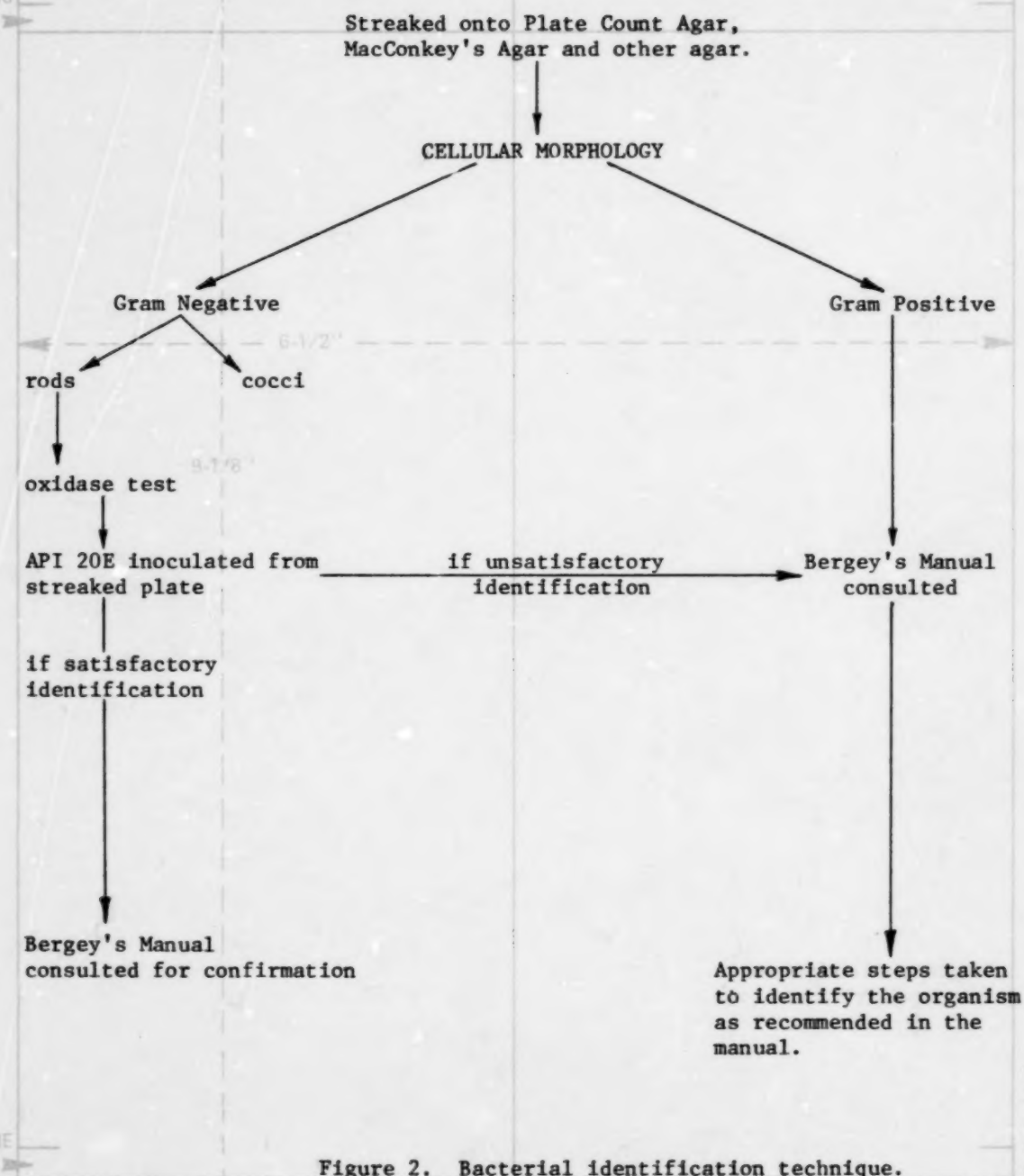

Streaked onto Plate Count Agar, MacConkey's Agar and other agar.
CELLULAR MORPHOLOGY
Gram Negative
Gram Positive
rods
cocci
oxidase test
API 20E inoculated from streaked plate
if unsatisfactory identification
Bergey's Manual consulted
if satisfactory identification
Bergey's Manual consulted for confirmation
Appropriate steps taken to identify the organism as recommended in the manual.

Figure 2. Bacterial identification technique.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

b. SPC increased when the turbidity increased.

4. SPC increased when coliform density increased.

5. Turbidity increased when the free chlorine residual increased.

6. Turbidity decreased when the sample temperature increased.

The second method, Program II, was also based on percentages. A typical test statement was "Test is positive if coliform count is 1/100 ml or greater (1/100 mL) when the free chlorine is 0.0 mg/L". The number of times this statement was correct would then be given as a percentage of the total number of times the test had a free chlorine residual of 0.0 mg/L. The analysis would then be modified by changing a single variable in the test statement, i.e., "Test is positive if coliform count is 1/100 mL or greater (1/100 mL) when free chlorine is 0.2 mg/L". This analysis would again be modified until all the possible combinations for that statement had been tested.

The statements and variables used in this method were as follows:

1. Test is positive if coliform count is 1/100 mL or greater (1/100 mL) when free chlorine is 0.0 mg/L.

   Variables a. Coliform count 1/100 mL; 5/100 mL.

   b. Free chlorine 0, 0.2, 0.5, 1.0 mg/L.

2. Test is positive if SPC 3/mL when free chlorine is 0.0 mg/L.

   Variables a. SPC 3, 10, 50, 500/mL.

   b. Free Chlorine 0, 0.1, 0.5, 1.0 mg/L.

3. Test is positive if coliform count is 1/100 mL when turbidity is 1.0 NTU.

   Variables a. Coliform count 1/100 mL; 5/100 mL.

   b. Turbidity 1.0; 1.00 NTU.

4. Test is positive if SPC 3/mL when turbidity 1.0 NTU.

   Variables a. SPC 3, 10, 50, 500/mL/

   b. Turbidity 1.0, 1.00 NTU.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

5. Test is positive if ≥0.2 mg/L free chlorine is present when turbidity is >0.60 NTU.

   Variables a. Free chlorine 0.2, 0.5, 1.0, 1.3 mg/L.

   b. Turbidity 0.60, 1.0 NTU.

6. Test is positive if coliform count ≥1/100 mL when SPC ≥3/mL.

   Variables a. Coliform count ≥1/100 mL; ≥5/100 mL.

   b. SPC ≥0, 3, 10, 50, 500/mL.

Program II was used to determine a total mean percentage over the entire span of the study. It was felt the parameter of temperature exerted the greatest influence on the growth of the microorganisms. Therefore, the data was broken up into blocks so this parameter could be analyzed separately, in conjunction with the above mentioned test statements. The temperature ranges chosen to determine this block of data were 1° to 7°C, 8° to 16° and 17° to 25°C. The time frames of the resulting divisions are listed below:

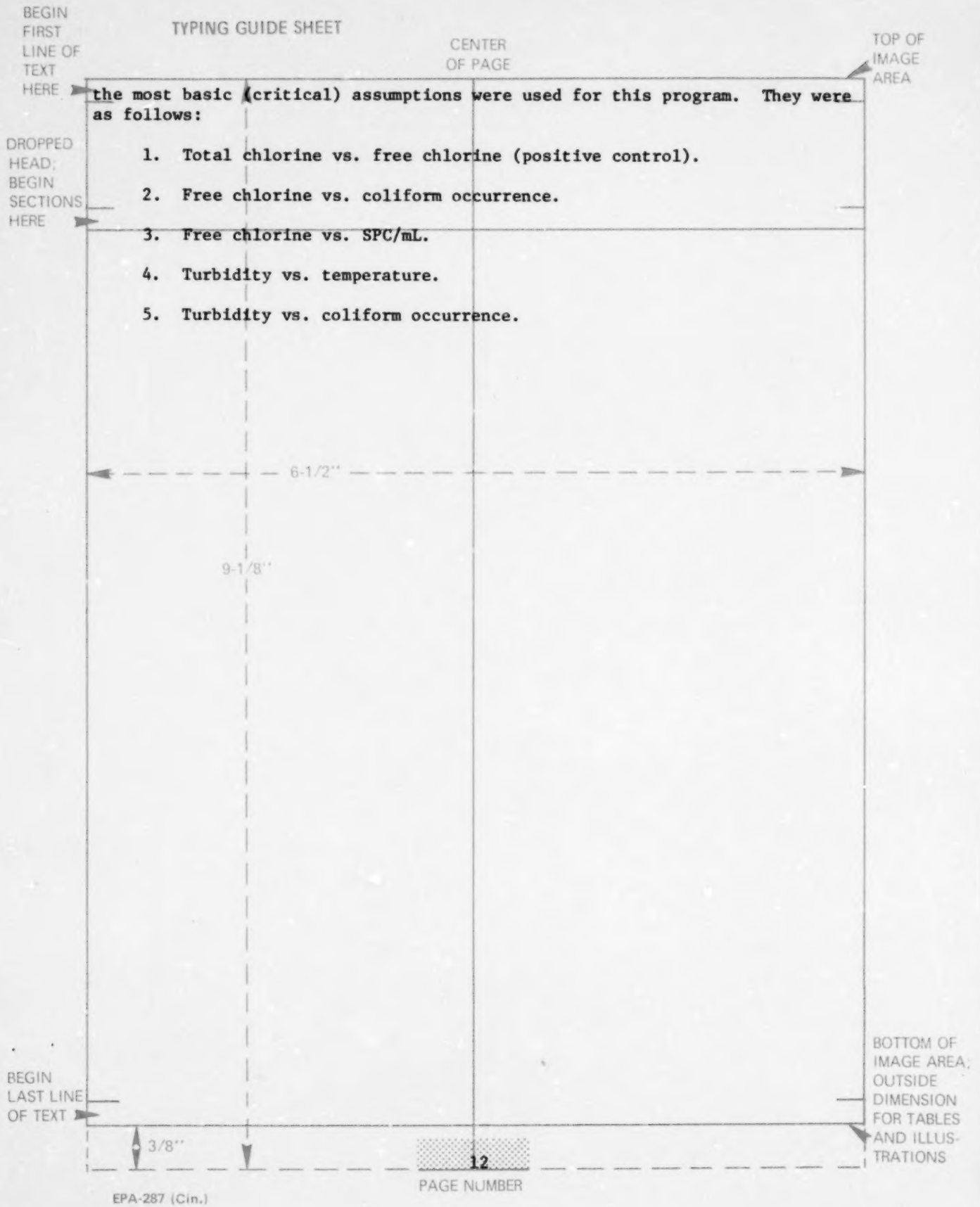
1. 11/1/76 - 12/14/76 (16° - 8°C).
2. 12/20/76 - 4/12/77 (7° - 1° - 7°C).
3. 4/19/77 - 6/14/77 (8° - 16°C).
4. 6/20/77 - 10/12/77 (17° - 25° - 17°C).
5. 10/17/77 - 12/19/77 (16° - 8°C).
6. 12/28/77 - 4/18/78 (7° - 1° - 7°C).
7. 4/24/78 - 6/12/78 (8° - 16°C).
8. 6/19/78 - 9/18/78 (17° - 25°C).

The results of Program II were examined to determine if there were significant differences or agreements in respect to the temperature parameters within the 8 zones as compared to the mean percentage of the overall project results of Program II.

Program III, a simple correlation coefficient (r), was derived between two parameters from which a coefficient of determination (r^2) was computed. Throughout the study there seemed to be an apparent lack of interrelationship between the parameters (physical, biological, and chemical) within the distribution system, as observed weekly, Therefore, to justify this visual observation it was determined that a correlation (r) analysis should be performed, especially on certain parameters where the data seemed to be contrary to some fundamental assumptions. The use of the coefficient of determination (r^2) was justified since the relationship between two parameters could be expressed as an absolute value percentage of correlation. Only

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cln.)
(4 76)


BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
the most basic (critical) assumptions were used for this program. They were as follows:
1. Total chlorine vs. free chlorine (positive control).
2. Free chlorine vs. coliform occurrence.
3. Free chlorine vs. SPC/mL.
4. Turbidity vs. temperature.
5. Turbidity vs. coliform occurrence.
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
12
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

SECTION 5

RESULTS AND DISCUSSION

PROGRAM I

The rationale behind Program I was to determine if certain generalizations accepted by most water utilities (either drawn from the conclusions of laboratory experimentation or from the general consensus of experience about distribution systems) were valid, when compared to actual data collected from established distribution systems over a lengthy period of time. A program was set up based on percentages to compare certain variables and their historical relationships. The first criterion to be met for test statement 1a was whether the free chlorine residual had increased from the previous week at that particular sampling point. If free chlorine residual had increased, then the coliform level was determined for the same test site. Table 1 shows the percentage of samples demonstrating bacterial reductions when free chlorine levels increased.

There are several nuances that should be pointed out about the wording and intended scope of Program I's test statement. First, the test was not designed to measure a quantitative reduction of the bacterial populations; it was designed to measure the percent samples where bacteria decreased when the free chlorine increased. Second, the test does not measure what amount of free chlorine was needed to reduce the size of the bacterial population. Third, it is important to note the phrasing "coliform density decreased or remained at 0". It was assumed for purposes of the test statement, that coliform densities were normally 0 or returned to 0 when the free chlorine residual increased from the previous week. This third statement establishes two possible conditions. First, when the free chlorine residual increased, coliform density should have remained at 0 or decreased in those samples where coliform were present the previous week. Secondly, when the free chlorine residual increased, coliform density should have increased from the previous week 0% of the time. The water industry, in general, would readily subscribe to both of these assumptions due to laboratory experiments demonstrating that free chlorine residuals will destroy coliform bacteria. (It should be noted here that pH, contact time and temperature in the Salem and Beverly distribution systems during the study, met criteria previously established by laboratory experimentation (39-45) to achieve an effective kill.) However, if it is assumed the free chlorine residual did not affect the increase or decrease of coliform levels, then the percentage of increase or decrease would be random event. Coliform densities would increase in 50% of the samples and decrease in the other 50%. A randomly distributed sample not affected by a free chlorine residual would form a normal curve and demonstrate the independence of coliform bacteria from the effects of a free

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.) (4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

chlorine residual.

With these options established, the data were analyzed and resulted in 82% compliance with test statement 1a, demonstrating that coliform densities increased in 18% of the samples when the free chlorine residual increased. This was obviously far from the 0-1% level demonstrated in laboratory disinfection experiments (39-45). A chi-square (χ^2) analysis was performed to determine if those percentages represented more than a random effect of free chlorine residual on coliform bacteria. Coliforms and free chlorine residuals are mutually exclusive categories which lend themselves very nicely to this type of analysis. A strict 0.1% (P=0.001) confidence limit was imposed for this particular test statement. The χ^2 analysis indicated that the decrease or increase in the coliform population was not due to chance. The implication was the free chlorine residual was responsible for a non-random decrease in the coliform population. However, the level of decrease did not meet the 100% expected kill level.

The results of the two different analyses (χ^2 and test statement 1a), may be resolved if it is kept in mind these results were from a working distribution system. In laboratory experiments the methods of analysis are carried out under highly controlled conditions. The conditions do not simulate the complex environment of a distribution system with its dead end mains, tubercles, silt, low flow areas and bacterial populations. Coliform bacteria used in the controlled experiments were probably of a single species and possibly a single biotype, thus ignoring whole groups of coliform bacteria that may be isolated from distribution systems. Therefore, isolation of coliforms should not be considered "stray" or "spurious", merely because they were recovered from water with a free chlorine residual. If, for the purposes of Program I, it is hypothesized that distribution systems have an established microbial flora, then the coliform bacteria may be an integral part of this flora. It is in this light the coliform density increase in 18% of the samples when the free chlorine residual increased may be understood. This microbiological flora hypothesis is dealt with more fully in the subsequent Microbiological Identification Section.

The arguments put forward for test statement 1a were designed not only to dissect their relationship but to demonstrate the same type of logic other variables were subjected to in Program I. Two of the test statements used in categorizing and analyzing data along with their results and confidence limits are given in Table 1.

TABLE 1. TEST STATEMENT 1. BACTERIAL DENSITY VS. FREE CHLORINE

Bacterial density decreased or remained at 0 when the free chlorine residual increased.

Item	Test Statement 1 a. Coliforms	Test Statement b. Standard Plate Count
% samples	82	65
Confidence limit	(P<0.001)	(P<0.010)

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-78)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

The reasoning used for 1a was directly applicable to test statement 1b. The SPC's were not reduced as effectively as coliforms by increased chlorine residuals. One hypothesis for this effect is the SPC bacterial populations were more diverse than the coliform group, resulting in a greater possibility of recovering organisms less susceptible to destruction by increasing free chlorine residuals. The lower percentage indicated a lesser degree of influence by the variable "free chlorine increased" in test statements 1a and b. A chi-square (χ^2) analysis performed on the above test statements demonstrated a strong association between all the test variables and free chlorine residuals, but the degree of interrelation of coliform reduction with increasing chlorine residuals was the strongest association.

The next 4 test statements evaluated the effects of distribution water temperature and turbidity on bacterial isolations from the distribution system. Tables 2 and 3 summarize the results of these analyses.

TABLE 2. TEST STATEMENT 2. BACTERIAL DENSITY VS. TEMPERATURE

Bacterial density increased when the water temperature increased.

Item	Test Statement 2 a. Coliforms	Test Statement b. Standard Plate Count
% samples	16	51
χ^2 Confidence limits	(P<0.001)	(P>0.050)

TABLE 3. TEST STATEMENT 3. BACTERIAL DENSITY VS. TURBIDITY

Bacterial density increased when the turbidity increased.

Item	Test Statement 3 a. Coliforms	Test Statement b. Standard Plate Count
% samples	16	48
χ^2 Confidence limits	(P<0.001)	(P>0.050)

It appears the SPC was independent of distribution water temperature and turbidity, whereas the coliform bacterial parameter apparently decreased or remained unchanged far more often than it increased. However, it must be restated, these results were from a working distribution system and other variables may have affected the final results of the test statements. Such was the case with the above test statements, and the key was the similarity of the results of the membrane filtration analysis in Tables 2 and 3 to those of Table 1. The maximum difference of only 3% between test statements 2 and 3

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.) (4-76)

lead one to conclude the interrelationships within the individual test statements were affected by a third dominant parameter, that of a free chlorine residual. The effect of this third variable was to skew the results of the test statements toward those of free chlorine residual test statement 1. The combined effect of the data in Tables 1-3 is to reveal that the primary influence in a distribution system is the free chlorine residual, not temperature or turbidity.

The remaining three test statements and their results are shown in Table 4.

TABLE 4. TEST STATEMENT 4, 5 and 6. SPC DENSITY VS. COLIFORM DENSITY, TURBIDITY VS. FREE CHLORINE, TURBIDITY VS. TEMPERATURE

Test Statement 4. SPC increased when the coliform density increased.	
% samples	32
Confidence limits	(P<0.001)
Test Statement 5. Turbidity increased when the free chlorine increased.	
% samples	35
Confidence limits	(P<0.010)
Test Statement 6. Turbidity decreased when the water temperature increased.	
% samples	40
Confidence limits	(P<0.050)

Results of test statement 4 indicate the SPC decreased or remained unchanged more often than it increased, when the coliforms increased. This conclusion supports previous work indicating SPC organisms suppressed coliform recovery on the membrane filter, although the SPC levels in these papers (1,16,32) were far greater ($\geq$500/mL) than the Standard Plate Counts experienced in this study. It is possible, however, that coliforms were suppressed by organisms the SPC did not enumerate. Victoreen (26) has demonstrated there are a great many microbes present in a water sample which escape detection by this method.

It was revealed in test statement 5 that as free chlorine residual increased, turbidity decreased. The increase in free chlorine residual may be interpreted as an indicator of "new" water from the treatment plant, which has not been in the distribution system a long time. Water with a longer residence time would be likely to have a higher turbidity due to alum floc deposition and a lower free chlorine level. It should be noted the authors did not expect this result. It was thought that an increase in free chlorine

residual would oxidize encrusted particles in the mains and result in significant increases in turbidity. This, however, did not occur.

Results of test statement 6 indicate a slight relationship between temperature and turbidity increases. Due to previous problems of alum breakthrough in cold water at the filtration plant and the observations of warmer water improving precipitation, flocculation, sedimentation and filtration processes at the plant, it was assumed turbidity would decrease with increasing sample temperature. Once again, the relationship determined by this test statement should be analyzed in the light of other parameters not taken into account in the test statement. In this case, it is plausible that the operating status of the filtration plant during the study played a determining factor in the test statement's result. The plant was operating on two of its four settling basins and on five of its eight filters at various times during the spring and summer months, when the water demand was either increasing or at its maximum. Presumably, alum breakthrough was occurring due to the severe hydraulic constraints placed on the plant during these periods of high consumption. Concomitant with this time frame, the ambient water temperature was rising. It is possible from these conditions to obtain the direct relationship of turbidity increasing as the sample temperature increased.

PROGRAM II

Program II differed from Program I by attempting to assess the variables in the distribution system by setting specific levels and determining what percentage of the sampled population was capable of meeting the imposed criteria. Test statement 1 of Program II exemplifies the difference; "test is positive if one or more (≥1) coliforms/100 mL are present when the free chlorine is ≥0.0 mg/L". The criteria of "≥1 coliform" and "free chlorine ≥0.0 mg/L" have been set and it must now be determined if the individual samples are able to meet the imposed standards of the test statement. The resulting percentage compliance reveals the frequency at which the test statement criteria were met.

The purpose of test statement 1 was to analyze what effective level of free chlorine was needed to control the presence of coliforms. The basis for determining test statement 1 criteria were as follows:

a. Coliform

   1. The calculated percentage of <1 coliform was derived from the ≥1 results and was included in the table for clarity.

   2. ≥1 coliform/100 mL level was selected to establish the presence of coliforms.

   3. ≥5 coliform/100 mL level was used to evaluate the Safe Drinking Water Act maximum contaminant level (MCL)(46).

b. Free Chlorine

   1. ≥0.0 mg/L was used as a baseline for the comparison of

higher chlorine levels.

2. ≥0.2 mg/L was selected on the basis of previous laboratory experiments having established this level as an adequate threshold of disinfection (34,35,39-45). The EPA also uses this level as a substitution for its monitoring requirements in the microbiological section of the Safe Drinking Water Act (46).

3. ≥0.5 mg/L level was chosen as a reasonable mid-range level between the "adequate" 0.2 mg/L and the 1.0 mg/L level.

4. ≥1.0 mg/L level was chosen because this level was reached in the distribution systems at various times and it would have to be considered more than capable of rendering most waters safe for drinking.

The results of test statement 1, Program II, are displayed in Table 5 along with different levels used for the variables. Note that Program II and III differ from Program I, since data from both cities are analyzed and displayed separately, as opposed to Program I which combined both distribution systems.

TABLE 5. TEST STATEMENT 1. EFFECT OF FREE CHLORINE ON COLIFORM DENSITIES IN TWO DISTRIBUTION SYSTEMS

		Free chlorine (mg/L)			
System	Coliform 100 mL	≥0.0 % samples	≥0.2 % samples	≥0.5 % samples	≥1.0 % samples
Salem	<1	78 (900)*	80 (336)	80 (176)	86 (80)
	≥1	22 (257)	20 (84)	20 (44)	14 (13)
	≥5	9 (107)	6 (27)	7 (16)	7 (5)
Beverly	<1	82 (863)	78 (291)	78 (106)	81 (13)
	≥1	18 (193)	22 (82)	22 (30)	19 (3)
	≥5	8 (60)	10 (37)	11 (15)	14 (3)

*The numbers in parentheses are the number of samples meeting the imposed test statement criteria.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

Inspection of Table 5 discloses that the frequency of coliform isolation was independent of the amount of free chlorine present in the sample at the time of collection. The sampled population that contained at least 0.0 mg/L free residual chlorine (effectively all samples in the study) and had coliforms isolated were the largest sampled group. With increasing free chlorine levels, the sample size population that met the required levels decreased and was a subset of the 0.0 mg/L sample population. Despite the smaller sample size with each increasing free chlorine level (read across) the frequency of coliform isolation did not significantly decrease. This occurred not only at the ≥1 coliform level but the MCL (≥5) as well. Further analysis of Table 5, reveals the frequency of coliform isolation decreases between the ≥1 level and the higher MCL of ≥5 (read down). When a free residual of ≥0.0 mg/L was present, coliforms were isolated from 22% and 18% of the samples from Salem and Beverly, respectively. Five or more coliforms were isolated from these systems in 9% and 8% of the samples at this same free residual level.

Test statement 2 was designed to analyze the effect of free chlorine on the SPC populations. The criteria for this test statement were as follows:

a. Standard Plate Count/mL.

1. The calculated percentage of <3 SPC was derived from the ≥3 results and was included in the table for clarity.

2. ≥3 SPC/mL level was chosen as a baseline.

3. ≥10 SPC/mL level was used because a large majority of the SPC were less than 10 colonies per plate.

4. ≥50 SPC/mL threshold was established from the visual observation that 95% or more of the plates had less than 50 colonies.

5. ≥500 SPC/mL threshold was established since it has been suggested this limit be used as a MCL in the Safe Drinking Water Act in various publications (1,16,32).

b. Free chlorine. Refer to test statement 1b, with the exception of the 0.1 mg/L level substituted in place of the 0.2 mg/L.

Table 6, Test Statement 2, again demonstrates that as the threshold level of the SPC variable increases (read down) the frequency of occurrence lessens due to the diminishing number of samples capable of meeting the imposed criteria in the test statement. It further reveals that the two distribution systems, although very old, are relatively "clean", at least as assessed by the SPC procedure. Most importantly, these results demonstrate a very definite reduction of the SPC with increased free chlorine residuals (read across), in contrast to the coliform results presented in Table 5. Increased chlorine residual levels from ≥0.0 mg/L to ≥0.1 mg/L effectively dropped the SPC percentage more dramatically in Salem than in Beverly. It may be speculated the older, more encrusted and slower flowing (higher retention time) Salem distribution system had a greater proportion of its pipe

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

TABLE 6. TEST STATEMENT 2. EFFECT OF FREE CHLORINE ON SPC DENSITIES IN TWO DISTRIBUTION SYSTEMS

		Free chlorine (mg/L)			
System	SPC/mL	≥0.0 % samples	≥0.1 % samples	≥0.5 % samples	≥1.0 % samples
Salem	<3	46	57	60	65
	≥3	54	43	40	35
	≥10	27	17	11	9
	≥50	8	4	2	1
	≥500	1	0	0	0
Beverly	<3	55	59	62	73
	≥3	45	41	38	27
	≥10	18	15	11	0
	≥50	5	4	2	0
	≥500	0	0	0	0

network harboring SPC organisms than the cleaner Beverly system. Historically, the newer Beverly system should have allowed higher levels of chlorine to travel throughout the distribution nerwork than in the Salem system, thus, allowing the chlorine to be more effective in the control of the microbial populations before this study was initiated. This would account for the lower percentage recorded for Beverly at the initial ≥0.0 mg/L free chlorine level. However, once the ≥0.1 mg/L level had been reached, both distribution systems had approximately the same percentages until the ≥1.0 mg/L level. Beverly's results deviated from Salem's at the ≥1.0 mg/L percent level, again on the lower side, probably due to Beverly's newer distribution system not allowing a suitable environment for the establishment of the microorganisms.

If this is correct, then why were the ≥0.1 mg/L and ≥0.5 mg/L results from Beverly similar to those of Salem? Possibly the threshold of "effective" disinfection in the "clean" Beverly distribution system was lower than it would have been in the older more encrusted Salem system. The "effective" level for Beverly was somewhere between 0.5 mg/L and 1.0 mg/L, where a sharp drop in the percentage was noted. Salem's "effective" level must have been higher than 1.0 mg/L because no such percentage reduction occurred between the ≥0.5 mg/L and ≥1.0 mg/L levels.

Test statement 3 was constructed to determine if low level turbidity

affected coliform recovery. For this test statement the criteria were as follows:

a. Coliform. Refer to test statement 1a.

b. Turbidity.

1. ≥1.0 NTU level was chosen to ascertain the "technical standard" of the 1 turbidity unit standard set in the Safe Drinking Water Act. Samples greater than 1.0 were not rounded to the nearest whole number. No turbidities were greater than 5 NTU.

2. ≤1.00 NTU parameter was chosen since the majority of samples were less than 1.00 NTU.

Test statement 4 was designed to reveal if different turbidity levels affected the SPC.

a. SPC. Refer to test statement 2a.

b. Turbidity levels. Refer to test statement 3b.

Tables 7 and 8, test statements 3 and 4, present the percent occurrence of coliforms and SPC bacteria at turbidity levels above and below 1.00 NTU. The tables reveal turbidity did not affect the frequency of occurrence of either coliform or SPC microorganisms. Similar results occurred for the frequency of coliforms and SPC at all levels using the variables of free chlorine (Tables 5 & 6) and turbidity (Tables 7 & 8). This inferred turbidity, of the type and at the levels experienced in these systems, did not affect chlorine disinfection.

TABLE 7. TEST STATEMENT 3. EFFECT OF TURBIDITY ON COLIFORM DENSITIES IN TWO DISTRIBUTION SYSTEMS

		Turbidity NTU	
System	Coliforms/100mL	≤1.00 % samples	>1.0 % samples
Salem	<1	77	78
	≥1	23	22
	≥5	9	7
Beverly	<1	81	83
	≥1	19	17
	≥5	10	5

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
6-1/2"
9-1/8"

TABLE 8. TEST STATEMENT 4. EFFECT OF TURBIDITY ON SPC DENSITIES IN TWO DISTRIBUTION SYSTEMS

		Turbidity NTU	
System	SPC/mL	≤1.00 NTU % samples	>1.0 NTU % samples
Salem	<3	47	44
	≥3	53	56
	≥10	25	30
	≥50	7	9
	≥500	0	1
Beverly	<3	53	57
	≥3	47	43
	≥10	18	17
	≥50	5	4
	≥500	0	0

In test statement 5, Table 9, an attempt was made to analyze if there was a relationship between free chlorine residual and turbidity. The criteria used for this test statement were as follows:

a. Free chlorine residual. Refer to test statement 1b, with the exceptions of deleting 0.0 mg/L and the addition of 1.3 mg/L level. The authors believed they had observed from time to time an increase in turbidity with increasing free chlorine residuals, hence the additional high chlorine variable.

b. Turbidity.

1. >0.60 NTU level was chosen because it was slightly lower than the observed average turbidity. A lower level was not selected since most of the samples did not achieve a lower turbidity level.

2. >1.0 NTU. Refer to test statement 3b.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.) (4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

Table 9. TEST STATEMENT 5. EFFECT OF TURBIDITY ON FREE CHLORINE IN TWO DISTRIBUTION SYSTEMS

System	Free Chlorine (mg/L)	Turbidity NTU 0.60 % samples	1.0 % samples
Salem	≥0.2	31	24
	≥0.5	16	10
	≥1.0	4	2
	≥1.3	2	1
Beverly	≥0.2	31	29
	≥0.5	9	7
	≥1.0	1	1
	≥1.3	0	0

The frequency of occurrence for all levels of free chlorine decreased as the turbidity levels increased from 0.60 to 1.0 NTU (read across) in Beverly, and to a greater degree in Salem. Why the discrepancy between the two systems? The answer to this may lie in the discussion section of test statement 2 where it was hypothesized the Salem system was not as "clean" as the Beverly distribution system. The larger decrease in the Salem system may have been due to the higher chlorine demand in the older Salem network while it was not as high in the newer, less encrusted Beverly system.

Test statement 6 was designed to analyze the relationship of the two microbial enumeration methods. The basis of test statement 6 criteria were as follows:

a. Coliform. Refer to statement 1a.

b. SPC. Refer to statement 2a, with the exception of adding the ≥0 parameter. This was added for greater delineation.

c. The figures in parentheses are the number of samples that met the imposed criteria for that appropriate category. They were included in order to demonstrate the validity of the conclusions drawn from this test statement.

Test statement 6 results are shown in Table 10. The coliform frequency was approximately the same throughout the SPC ranges of 0, 3, 10 and 50.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

TABLE 10. TEST STATEMENT 6. EFFECT OF SPC DENSITIES ON COLIFORM DENSITIES IN TWO DISTRIBUTION SYSTEMS

		SPC /mL				
System	Coliform 100mL	≥0 % samples	≥3 % samples	≥10 % samples	≥50 % samples	≥500 % samples
Salem	<1	78 (900)*	74 (521)	72 (242)	71 (64)	75 (18)
	≥1	22 (257)	26 (183)	28 (94)	29 (26)	25 (6)
	≥5	9 (107)	11 (73)	14 (42)	10 (7)	0 (0)
Beverly	<1	82 (863)	81 (431)	79 (147)	75 (45)	75 (6)
	≥1	18 (193)	19 (101)	21 (39)	25 (15)	25 (2)
	≥5	6 (60)	10 (39)	10 (17)	12 (4)	25 (1)

*The numbers in parentheses are the number of samples meeting the imposed test statement criteria.

The 500 level may be statistically invalid since only 24 samples in Salem and 8 in Beverly exceeded the 500 level. The table reveals three conclusions. First, the SPC levels do not affect the frequency of coliform recovery (read across). Secondly, the fact that coliforms appear with the same frequency, regardless of SPC levels, strongly suggests that the coliforms are a part of the distribution system's microbiological flora. This phenomenon is more fully dealt with in the Microbiological Identification Section. The third conclusion is that high or low SPC densities do not indicate either the presence or absence of coliform organisms.

Program II was further refined by delineating all six test statements into blocks of data that were separated on the basis of temperature. Temperature was assumed to be the most variable environmental parameter (other than residual chlorine) that would influence the relationship between two other test parameters. The blocks were composed of the temperature ranges from 1-7°C, 8-16°C and 17-25°C. These temperature ranges were selected to determine their effect on microbial population growth within the distribution systems. The division of 1-7°C was chosen due to the observed decrease of microbial activity in this range. Eight to 16°C was chosen on the basis of observing increased recovery of microorganisms at 8°C when moving into warmer temperature periods. The 16°C limit was determined by observing an

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

TABLE 11. TEST STATEMENT 4. EFFECT OF TEMPERATURE ON SPC AND TURBIDITY

System	SPC/mL	+ Turbidity NTU	% of samples meeting the stated conditions at these temperature ranges (C°)								Average
			8-16°	1-7°	8-16°	17-25°	8-16°	1-7°	8-16°	17-25°	
Salem	<3	>1.0	50	36	42	11	52	66	72	18	44
	≥3	>1.0	50	64	58	89	48	34	28	82	56
	≥10	>1.0	29	30	31	54	31	14	22	64	30
	≥50	>1.0	17	5	7	14	17	4	3	27	9
	≥500	>1.0	0	0	1	0	10	0	0	0	1
Salem	<3	≤1.00	48	45	46	27	55	64	51	54	47
	≥3	≤1.00	52	55	54	73	45	36	49	46	53
	≥10	≤1.00	27	26	19	32	20	18	26	37	25
	≥50	≤1.00	4	5	4	13	3	6	10	6	7
	≥500	≤1.00	0	0	0	1	1	1	0	0	0
Beverly	<3	>1.0	67	55	45	39	54	78	47	44	57
	≥3	>1.0	33	45	55	61	46	22	53	56	43
	≥10	>1.0	10	15	23	26	16	9	31	28	17
	≥50	>1.0	5	3	0	2	3	4	8	16	4
	≥500	>1.0	0	1	0	0	0	0	2	0	0
Beverly	<3	≤1.00	61	52	58	42	55	67	47	57	53
	≥3	≤1.00	39	48	42	58	45	33	53	43	47
	≥10	≤1.00	18	19	14	23	16	12	16	30	18
	≥50	≤1.00	7	6	3	8	2	5	9	0	5
	≥500	≤1.00	2	0	0	0	0	1	0	0	0

NOV. DEC. APR. JUNE OCT. DEC. APR. JUNE SEPT.

MONTHS

BEGIN LAST LINE OF TEXT
3/8"
EPA-287 (Cin.) (4-76)
PAGE NUMBER 25
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

apparent decrease in microbial recovery when moving downward from the 17-25°C temperature range. The 17°-25°C range appeared to represent the zone of greatest microbial recovery. It was assumed the low temperature range would be somewhat bacteriostatic to mesophilic organisms and there would be decreased recovery of these organisms as compared to recoveries at higher temperatures.

Test statements 5 and 6 did not demonstrate any significant influence by these temperature divisions. In test statements 1, 2, 3 and 4, there was no clearly definable increase or decrease in the percentages between the temperature blocks of 1-7°C and 8-16°C, as compared to the percentage fluctuations moving into and out of the 17-25°C range. In test statements 1 and 3 where the coliform variable was used, a pronounced increase and decrease in coliform recovery was encountered moving into and out of the first 17-25°C range. A second 17-25°C range, one year later, exhibited no influence by this temperature block due to an abatement of coliform bacteria at that time. Test statements 2 and 4, which dealt with SPC bacteria, demonstrated the greatest temperature influence. Only the results of test statement 4 are presented in Table 11 because of the similarity of results to those of test statement 2. It was apparent the fluctuation in percentages moving into and out of the lower temperature block was not as pronounced as it was moving into and out of the higher temperature blocks thus indicating increased activity of mesophilic microorganisms due to warmer temperatures. This observation was valid throughout most of the SPC and turbidity levels of Table 11.

An attempt was made to gauge the possible changes in species diversity of SPC organisms recovered from the microbial ecosystem with respect to the temperature blocks. Outside of the corresponding increase in overall population of organisms at warmer temperatures, it could not be determined if there was any appreciable increase or decrease in any species between the temperature blocks. However, the inability to isolate any predominating organisms that were temperature dependent may have been due to the random selection of organisms picked from the SPC. Also, the small number of organisms identified as compared to the much larger number of organisms isolated by the plates could give an inappropriate profile of the microbial population within the temperature blocks.

PROGRAM III

The results of Program III's coefficient of determination (r^2) are listed in Table 12. The r^2 values were assigned into 0.100 unit increments and a percentage of these values were determined, based on an 89 week period. This was done to ascertain the range of r^2 values with respect to each other. Over eighty-eight percent of the weekly r^2 values (free chlorine vs. coliform, free chlorine vs. SPC, turbidity vs. temperature and turbidity vs. coliform) were below 0.500 r^2 value. This r^2 value was established defining a limit where $\geq$0.500 units of the value was due to covariance, while <0.500 units was due to random chance. The majority of the r^2 values indicated very little co-variance ($\geq$.500 r^2) between the respective test parameters. This demonstrated the results in a working distribution system were affected by multiple influences not found within the strict controls imposed by laboratory

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

TABLE 12. COEFFICIENT OF DETERMINATION FOR FREE CHLORINE, TOTAL CHLORINE, COLIFORM, SPC, TURBIDITY AND TEMPERATURE

System	r^2	% of Samples: Total chlorine vs. free chlorine	Free chlorine vs. coliform	Free chlorine vs. SPC/mL	Turbidity vs. temperature	Turbidity vs. coliform
Salem	.000-.099	3.4	75.3	72.4	68.2	76.4
	.100-.199	0.0	13.5	21.8	8.2	12.4
	.200-.299	0.0	3.4	2.3	11.2	2.2
	.300-.399	0.0	1.1	1.1	7.1	3.4
	.400-.499	2.2	1.1	1.1	0.0	3.4
	.500-.599	1.1	1.1	0.0	4.7	1.1
	.600-.699	0.0	2.2	0.0	0.0	0.0
	.700-.799	4.5	1.1	0.0	0.0	0.0
	.800-.899	6.7	0.0	0.0	0.0	1.1
	.900-1.00	82.0	1.1	1.1	0.0	0.0
Beverly						
	.000-.099	7.0	77.3	71.8	64.6	77.5
	.100-.199	1.2	11.5	17.6	9.2	12.4
	.200-.299	1.2	3.4	5.9	13.8	5.0
	.300-.399	3.5	3.4	1.2	7.8	3.4
	.400-.499	2.3	1.1	2.4	1.5	1.1
	.500-.599	7.0	2.3	0.0	3.1	0.0
	.600-.699	5.8	0.0	1.1	0.0	1.1
	.700-.799	14.0	0.0	0.0	0.0	0.0
	.800-.899	23.3	0.0	0.0	0.0	0.0
	.900-1.00	34.9	1.1	0.0	0.0	0.0

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

experimentation. The only correlation that did have a high majority of its r^2 values over 0.500 was the total chlorine vs. free chlorine analysis. This result confirms the assumption that the total and free chlorine residuals covary in respect to each other (the free chlorine residual making up part of the total chlorine value). This phenomenon was evident on a weekly observational basis and it was used as a positive control to confirm that the program was operating correctly.

RAW AND FINISHED WATER PARAMETERS

Raw and finished water quality were monitored at the filtration plant in order to determine if the plant was being severely overloaded by its source water. Also, this provided baseline information on the quality of water supplied to the distribution systems. Data for the raw and finished water are listed in Table 13. Clearly, the raw water was always good quality and presented no problems for the treatment system. Good quality water was delivered consistently to the two distribution systems.

TABLE 13. RAW AND FINISHED WATER PARAMETERS

Item	Turbidity NTU	Coliforms/100mL MF	Coliforms/100mL MPN	SPC/mL	pH	Free chlorine mg/L
Raw water entering plant:						
Average	1.8	83	101	57	6.64	--
High	2.7	500	2400	TNTC	7.59	--
Low	0.7	<1	<2	<1	6.20	--
Finished water leaving plant:						
Average	0.59	<1	<2.2	3	7.04	1.7
High	1.0	<1	5.1	10	7.80	3.1
Low	0.24	<1	<2.2	<1	5.96	0.4

PARAMETER RANGES AND THOSE NOT ANALYZED

pH's measured throughout the study were not used in the final analysis of the data. pH did not appear to influence other parameters measured in this study, probably due to the narrow range it was kept within during most of the study period, although the range in Table 14 does not appear to reflect it. The majority of the samples (85%) were in a range of 6.58 to 7.42 with a mean of 6.99. The other parameters used in this study are presented in Table 14. Also, two spotcheck MPN samples, done weekly on each distribution system, were dropped from the final analysis due to the high percentage of agreement between the MPN and membrane filtration methods. The majority of the samples were negative for coliforms, hence the high agreement. However, when coliforms were present, the two methods agreed both on coliform

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

TABLE 14. PARAMETER RANGE OF DISTRIBUTION SYSTEM SAMPLES*

Item	Turbidity NTU	Coliforms/ 100 mL MF	SPC/mL	pH	Free chlorine mg/L	Temp. °C
Salem and Beverly						
Average	1.2	2.8	23	6.99	0.2	12
High	115	400	3000	8.12	2.3	24
Low	0.16	0	0	5.77	0	0

*Based on a total of 2,213 samples.

presence and approximate density. Due to the high degree of accuracy (95%) between the two methods, it was determined that no useful purpose would be served in analyzing the MPN results against the other parameters measured in the study.

6-1/2"

CONFIRMATION PROCEDURE

The confirmation procedure was used throughout the study for all samples that recovered typical sheen colonies. Out of the 742 typical sheen colonies picked from distribution samples for the confirmation procedure, 62% (461) were positive and 38% (281) were negative. In addition, Gram stains (Completed Test) and inoculation of API 20E identification strips were performed with the same sheen colonies used in the confirmation procedure. All of these organisms were determined to be Gram negative rods, regardless of whether they were positive or negative in the confirmation procedure. Also, many confirmed negative colonies were later identified as members of the family Enterobacteriaceae. Due to the inconsistency of the confirmation procedure when compared to the Gram stains and API system, it was determined the confirmation procedure was not suited for the purposes of our analyses. Therefore, total sheen colony counts were used in the final analyses of the results.

BEVERLY'S REVERSAL OF FLOW

One anomaly of the project occurred in the Beverly distribution system beginning November 4, 1977 and continuing for the next five days. Through an error in communications on a Friday afternoon, a small, isolated high service area was opened to an adjacent low pressure service area, which in turn was connected to the rest of the Beverly system. The high service areas' average water consumption for a weekend was 800 thousand liters (211 thousand gallons) and that of Beverly as a whole was 40 million liters (10.6 million gallons) for the same period of time. During that weekend, 8 million liters (2.1 million gallons) were pumped through the high service area into the rest of the Beverly distribution system, thus contributing 21% of the water consumption. The rerouting of the water either completely reversed or greatly altered the flow patterns in Beverly before the error was corrected on the following Monday morning.

BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
BEGIN LAST LINE OF TEXT
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

Unaware of this weekend occurrence, the Beverly and Salem distribution system samples were collected and processed that Monday as usual. The turbidities in Beverly (range 0.87 to 2.1 NTU) averaged higher than the 1.0 NTU average of Salem. The free chlorine residual in Beverly ranged from 0.2 mg/L to 0.9 mg/L, while Salem's chlorine residual range was from 0.0 mg/L to 1.0 mg/L. The total coliform count ranged from 1 to 125/100 mL in 11 of the 12 regular sampling points in Beverly, while Salem had only 1 coliform isolate. Many of the sampling points in Beverly had the direct relationship of low chlorine-low coliform and high chlorine-high coliform. Resampling was immediately instituted in Beverly upon discovery of the coliform results and an investigation was begun as to the possible cause of this sudden coliform occurrence. Negative coliform results were recorded for the next two repeat sampling days. The coliform organisms isolated that Monday from the Beverly samples were identified as Enterobacter cloacae and Enterobacter agglomerans. These organisms had been isolated in both distribution systems previous to this reversal of flow.

The filtration plant was functioning properly during that weekend period, otherwise the Salem system would have exhibited similar sampling results as those in Beverly. Also, once the change in flow patterns in Beverly had been corrected the system returned to "normal", which again indicates the filtration plant was operating properly. Therefore, coliform organisms must have been present in the distribution system network.

If it is theorized microorganisms utilize nutrients from the pipe environment, then it is reasonable to assume the disruption of this cycle would lead to the subsequent shedding of organisms into the water if that flow of nutrients were reversed and no longer available. The physical phenomena of sudden increased pressure and sheer forces due to the altered flow patterns must have "scoured" the pipes to some extent, as was evidenced by the increased turbidity levels, thus removing some organisms from their habitat sites in the pipes. In addition, a chemical phenomena may have played a part in the release of coliform organisms. The introduction of increased free chlorine residuals into areas that did not normally receive that magnitude of a residual could have oxidized and subsequently released turberculation along with coliform organisms into the distribution system. Why, if all of the above statements were correct, did the SPC not increase concomitantly with the coliform bacteria? A possible twofold answer to this question lies in the capsular structure surrounding environmental coliforms and the suppression of coliforms by other microorganisms. It is possible with the introduction of increased free chlorine levels the coliform population, that had been previously suppressed by antagonistic organisms, was no longer suppressed, due to the inability of antagonistic microorganisms to cope with the bactericidal effects of free chlorine. More specifically, coliform organisms were able to produce a capsule, which inhibited the effects of a free chlorine residual. This conferred upon those coliform bacteria a selective advantage of surviving in the new increased free chlorine residual environment created by the reversal of flow in Beverly. It is in this manner the seeming anomaly of increased coliforms and decreased plate counts may be resolved into a rational event.

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

The ability of environmental microorganisms, including coliforms, to protect

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA

themselves from the stresses encountered within a distribution system are dealt with more fully in the Microbiological Identification Section.

DROPPED HEAD; BEGIN SECTIONS HERE

CHLORINE/COLIFORM EXPERIMENTS

The reversal of flow incident set in motion four rudimentary laboratory experiments designed to demonstrate the ability of coliform organisms to survive in chlorinated tap water, thereby corroborating the reversal of flow phenomenon in Beverly and the less dramatic isolations of coliforms observed weekly in both distribution systems. Klebsiella pneumoniae, Enterobacter cloacae and Enterobacter agglomerans, isolated from the distribution systems, were used in these experiments. The scope and intent of the experiments varied from a modest attempt to determine if an encapsulated coliform could survive in chlorinated tap water (0.5 mg/L, total) for a short duration (15 seconds to 1 hour), to the more complex task of determining when coliform organisms succumb to the effects of chlorination, while following the concomitant decrease of chlorine residual during that time interval. Capsule stains were done in all experiments and the organisms were observed to have a capsule present before and after the different experiments. The pH range in all the experiments was between 6.7 and 7.0. The results of the experiments were mixed and their individual presentation would be cumbersome and confusing. However, several conclusions may be extracted from the body of the material. First, the minimum survival time of encapsulated organisms in these experiments was 5 hours in tap water at 3°C and 21°C, whose initial free chlorine residual of 1.2 mg/L was allowed to deteriorate during the course of the experiment. Second, an Enterobacter cloacae strain that lacked a capsule and an encapsulated Enterobacter cloacae were used in one other rudimentary experiment. The encapsulated coliform was able to survive in 1.5 mg/L free chlorine residual tap water for 48 hours, while the nonencapsulated coliform organism was not. The free chlorine residual was allowed to deteriorate during the course of this experiment. The overriding conclusion of all four experiments supported the weekly observation from regular distribution system samples and the reversal of flow incident in Beverly, that viable coliform organisms had the capability of being recovered from chlorinated tap water.

The foregoing discussion should not be construed to declare a free chlorine residual does not kill coliform bacteria or disinfect the water; it does, as has been demonstrated in many laboratory experiments and distribution systems. However, it is obvious from the results of the data that it does not destroy all the coliform bacteria present in water distribution systems.

MICROBIOLOGICAL IDENTIFICATION

The diversity of organisms isolated from the distribution systems (Table 15) strongly suggests these organisms have established an ecosystem within those pipe networks. The ability of organisms, including coliforms, to establish and maintain this ecosystem is not surprising when it is realized the environment and the genetic potential of the microorganisms combine to form an ideal habitat for bacteria. It is in this light the results and conclusions of Program 1, 2 and 3 may be understood. If the distribution systems have a diversified microbial flora consisting of the organisms in Table 15 with their

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.) (4-76)

TABLE 15. MICROBIOLOGICAL IDENTIFICATION OF ORGANISMS ISOLATED USING THE MEMBRANE FILTER PROCEDURE FOR COLIFORMS AND THE STANDARD PLATE COUNT

Organism	Membrane filter isolates				Standard Plate Count isolates	
	Background		Sheen colonies			
	No. of isolates	% of total	No. of isolates	% of total	No. of isolates	% of total
Gram-Negative Aerobic Rods and Cocci						
Pseudomonas spp	5	13.2	0		20	7.5
Pseudomonas matlophilia	0		0		10	3.7
Pseudomonas mallei	0		0		2	0.7
Pseudomonas vesiculare	0		0		5	1.9
Pseudomonas aeruginosa	1	2.6	0		5	1.9
Pseudomonas fluorescens	0		0		7	2.6
Pseudomonas putida	0		0		2	0.7
Pseudomonas cepacia	0		0		7	2.6
CDC Group 11 K-2	0		1	0.3	0	
CDC Group 11 K-1	0		0		1	0.4
CDC Group 11 F	0		0		2	0.7
CDC Group UE 1	0		0		2	0.7
CDC Group 11 K	0		1	0.3	0	
Xanthomonas spp	0		0		2	0.7
Azotobacter spp	0		0		1	0.4
Rhizobium	0		0		5	1.9
Alcaligenes spp	0		0		4	1.5
Achromobacter xylosoxidans	0		0		1	0.4
Achromobacter spp	0		0		1	0.4
Gram-Negative Facultatively Anerobic Rods						
Escherichia coli	1	2.6	22	7.1	2	0.7
Citrobacter freundii	0		15	4.9	1	0.4
Klebsiella pneumonia	3	7.9	37	12.0	7	2.6

BEGIN LAST LINE OF TEXT
3/8"
EPA-287 (Cin.) (4-76)
PAGE NUMBER 32
BOTTOM OF IMAGE AREA. OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
DROPPED HEAD: BEGIN SECTIONS HERE
BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA

TABLE 15. (continued)

Organism	Membrane filter isolates: Background No. of isolates	Background % of total	Sheen colonies No. of isolates	Sheen colonies % of total	Standard Plate Count isolates: No. of isolates	Standard Plate Count isolates: % of total
Klebsiella ozaenae	0		1	0.3	0	
Enterobacter agglomerans	7	18.4	85	27.6	9	3.4
Enterobacter aerogenes	2	5.2	17	5.5	1	0.4
Enterobacter cloacae	9	23.7	111	36.0	6	2.2
Enterobacter hafnia	1	2.6	0		1	0.4
Serratia liquefaciens	0		2	0.6	2	0.7
Serratia marcescens	0		2	0.6	4	1.5
Proteus mirabilis	1	2.6	1	0.3	0	
Proteus morganii	2	5.2	2	0.6	1	0.4
Vibrio alginolyticus	0		0		1	0.4
Aeromonas hydrophila	1	2.6	6	1.9	8	3.0
Plesiomonas shigelloides	0		1	0.3	0	
Chromobacterium spp	0		0		10	3.7
Flavobacterium spp	0		0		17	6.3
Flavobacterium aquatilen	0		0		1	0.4
Acintobacillas spp	0		0		1	0.4
Gram-Negative Cocci and Coccobacilli						
Moraxella spp	2	5.2	0		5	1.9
Acinetobacter calcoaceticus	3	7.9	4	1.3	19	7.1
Acinetobacter antiratus	0		0		5	1.9
Neisseria spp	0		0		1	0.4
Gram-Negative, Chemolithotrophic Bacteria						
Nitrococcus spp	0		0		2	0.7

TABLE 15. (continued)

Organism	Membrane filter isolates: Background No. of isolates	Background % of total	Sheen colonies No. of isolates	Sheen colonies % of total	Standard Plate Count isolates No. of isolates	Standard Plate Count isolates % of total
Nitrosomonas spp	0		0		1	0.4
Thiobacillus spp	0		0		1	0.4
Ichrobium spp	0		0		1	0.4
Gram-Positive Cocci						
Micrococcus spp	0		0		9	3.4
Micrococcus luteus	0		0		3	1.1
Staphyloccus spp	0		0		4	1.5
Staphyloccus aureus	0		0		9	3.4
Staphyloccus pyogenes	0		0		1	0.4
Planococcus spp	0		0		2	0.7
Streptococcus faecalis	0		0		1	0.4
Sarcina lutea	0		0		2	0.7
Endospore-Forming Rods And Cocci						
Bacillus spp	0		0		13	4.9
Bacillus sublilis	0		0		2	0.4
Bacillus cereus	0		0		1	0.7
Clostridium spp					5	1.9
Sporolactobacillus	0		0		1	0.4
Actinomycetes And Related Organisms						
Corynebacterium spp	0		0		4	1.5
Arthrobacter spp	0		0		6	2.2
Actinomyces spp	0		0		8	3.0

TABLE 15. (continued)

Organism	Membrane filter isolates: Background No. of isolates	Background % of total	Sheen colonies No. of isolates	Sheen colonies % of total	Standard Plate Count isolates No. of isolates	Standard Plate Count isolates % of total
Mycobacterium spp	0		0		1	0.4
Streptomyces	0		0		4	1.5
Micromonospora	0		0		1	0.4
Sheathed Bacteria						
Leptothrix	0		0		3	1.1
Crenothrix	0		0		2	0.7
Other						
Desulfovibrio	0		0		1	0.4
Yeasts					numerous isolates	
Molds					" "	
Lactobacillus	0		0		1	0.4
Total	38		308		268	

BEGIN LAST LINE OF TEXT
DROPPED HEAD. BEGIN SECTIONS HERE
BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
EPA-287 (Cin.) (4-76)
3/8"
PAGE NUMBER
35
CENTER OF PAGE
BOTTOM OF IMAGE AREA. OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
TOP OF IMAGE AREA

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

varied characteristics then that flora can not and should not be expected to react in the same manner to one parameter (i.e. free chlorine residual or turbidity) with any sort of consistency. These organisms, including coliforms, are able to survive a multitude of physical, chemical and biological phenomena by means of their encapsulation (glycocalyx). This glycocalyx, a polysaccharide capsular coat that is generally absent from the pure laboratory cultures but not from environmental strains, enables the various environmental organisms to protect themselves from the "hostile" conditions of a distribution system.

Table 15 contains a list of soil and aquatic microorganisms isolated from the distribution systems during the study period. These include heterotrophic and chemolithotrophic energy metabolizers as well as nitrogen fixing bacteria. The diversity of microbial organisms isolated and the widely differing biochemical mechanisms employed by each species, certainly implies that an established flora is sustained within the distribution systems. Assuming that a microbial ecosystem has been established implies that environmental sources of energy have been modified and supply a flow of nutrients between species in order to support a diversified flora.

6-1/2"

Microbial growth within a distribution system is finite since there are a limited number of suitable microhabitats in which the proper biotic and abiotic parameters are present that enable the survival of a specific species or population. The role of a biological niche in a distribution system cannot be regarded as a steady state system, but rather should be considered in dynamic equilibrium, due to the ingress and egress of individual substances required for a given organism or population. (The species abundance of organisms is inherently limited to those microhabitats that encounter minimal competition with other species as well as having optimal physiochemical conditions for growth).

9-1/8"

Trace substances (primary substances) that escape the physiochemcial processes of a treatment facility could supply a source of energy (trace organics and other nutrients) to that portion of the microbial populations that is able to metabolize them (19). Certain types of bacteria growing at the pipe-water interface could selectively absorb either these primary substances present in the treated water or other metabolic products from other species or groups of organisms upstream. The primary substances would be transformed into secondary metabolites. The metabolic by-products excreted would diffuse downstream, possibly to another group of organisms which might absorb these secondary metabolites for use in their particular biochemical mechanism. Once the primary substrates have been converted to more complex metabolites then it can be assumed that neighboring biological niches provide a nutrient exchange by allowing for the growth of more fastidious organisms.

This is further supplemented by the microorganisms themselves, which contribute to the available energy supply stored within the ecosystem. If a particular species is unable to survive due to some sort of environmental change, then that dying species would become a nutrient source, due to the energy stored in biomass. Its death could possibly open up a new niche for other microbes in the biochemical food chain of the distribution system flora. Whatever the nutrient chain is, the main body of nutrient transfer would be

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

carried out at the pipe-water interface where the flow is minimal (47). Even in the high flow zones where protruding tubercles exist, the diluted nutrients are capable of being collected by the microbes as evidenced by the millions of organisms harbored in these nodules (28).

Coliform organisms are no different from other groups of microorganisms listed in Table 15, with regard to their capability of sustaining and replicating within the distribution system. They are able to replicate in water with trace organics present (48), as evidenced by the fact that 22.4% of the microorganisms randomly selected from the SPC during this study were coliforms. The coliform group makes up a rather remarkably large percentage of the SPC population identified and enumerated in Table 15. Although, taken in the context of the large number of coliforms isolated throughout the study period by the membrane filtration method and the hypothesis that these organisms comprise part of the ecosystem in the distribution system, it is not a remarkably large percentage and it is in fact a normal phenomenon that might be predicted. The majority of microbes listed in Table 15 (including the coliforms) have been isolated from drinking water by other investigators (1-32, 49-56). With this kind of documentation by different researchers, spanning over a thirty year period, discovering the same or different organisms living within a whole range of distribution systems, it must be concludedthat bacteria have adapted to ecosystems unique to their own situations. The variety of microbes isolated and identified from the SPC method in this study certainly suggests and points to that hypothesis. With a microbiological flora established throughout the distribution system, the generally independent nature of the results concluded for coliform and SPC populations when compared to temperature, turbidity and chlorine may be understood. The best quality water may be produced at a treatment facility and a high chlorine residual may be employed throughout the delivery system, but if the distribution system has microbial habitats established throughout its network then regardless of that high quality chlorinated water, microorganisms from that flora, including coliforms, may be isolated from that system.

How is it possible the environmental microorganisms are able to accomplish all these feats, such as not being swept away in the rapidly flowing water, collecting nutrients from the environment, symbiosis between colonies, and being able to withstand high doses of free chlorine? A principal answer to these questions comes from the observation that the coliforms isolated in the study had a polysaccharide capsule present. In environmental organisms the polysaccharide capsular coat is referred to as a "glycocalyx". It has been demonstrated that many soil and aquatic bacteria are surrounded by this cellular structure (57). Also, many strains of Enterobacteriaceae have the genetic potential and metabolic mechanisms to produce a glycocalyx, if certain environmental conditions have been met (58). It has been previously demonstrated that glycocalyx production has been induced at temperatures below optimum growth (59). This coat is usually absent from the pure laboratory culture used in controlled experiments. The lack of the glycocalyx in lab cultures greatly alters the conclusions drawn from these experiments, when those conclusions are extrapolated to organisms in an environmental situation. "The glycocalyx is essential to the biological success of most bacteria in the varied natural environments in which they are observed" (60). The fibers of the glycocalyx are negatively charged and seek divalent cations to mediate the

BEGIN LAST LINE OF TEXT HERE
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
9-1/8"
BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
PAGE NUMBER
EPA-287 (Cin.) (4-76)

anchoring of themselves to a surface in order for the bacterium not to be carried away in the flowing water. Obviously, the iron pipes are more than capable of supplying this type of base. Once a single bacterium has anchored itself, it may then build on itself forming microcolonies. Other species may enter the glycocalyx of the microcolony and establish themselves if the relationship is beneficial to the participants. A common glycocalyx (more than one species within the glycocalyx)can serve as a food reservoir whose dead cells are then recycled by the surviving cells (60). This microcolony may supplement its own glycocalyx food reservoir by binding nutrients, ions and molecules that enter into the environment surround it, somewhat in the manner of an ion exchange resin (61). The glycocalyx not only binds useful material it "traps even small harmful ions and molecules in the environment" (60). The implication is clear, the glycocalyx has not only the physical barrier capacity of the dense polysaccharide coat to protect itself from free chlorine molecules and ions but the chemical capability as well. The observation of the presence of a capsule on almost all of the coliforms isolated from the distribution systems turned out to be one of the major turning points in this study. The presence of a capsule, glycocalyx, confers upon the microbe not only the ability to "stick" to pipes in moving water, and collect nutrients from the environment around itself, but to lessen the effectiveness of the disinfectant chlorine. With the "neutralization" of free chlorine, the water utility's primary defense mechanism, the distribution system becomes an ideal environment for the survival and growth of microorganisms.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

References

1. Geldreich, E.E., Nash, H.D., Reasoner, D.J. & Taylor, R.H., The Necessity of Controlling Bacterial Populations in Potable Water. Jour. AWWA, 64:9 (1972).

2. Walton, G., Effectiveness of Water Treatment Processes as Measured by Coliform Reduction. PHS Pub., 898 (1961).

3. Tracy, H.W., Camarena, V.M. & Wing, F., Coliform Persistence in Highly Chlorinated Water. Jour. AWWA, 58:9 (1961).

4. Sen, R. & Jacobs, B., Pathogenic Intestinal Organisms in the Unfiltered Water Supply of Calcutta and the Effects of Chlorination. Ind. Jour. Med. Res., 57 (1969).

5. Chang, S.L., Berg, G., Clarke, N.A. & Kabler, P.W., Survival and Protection Against Chlorination of Human Enteric Pathogens in Free Living Nematodes Isolated from Water Supplies. Am. Jour. Trop. Med. Hyg., 9 (1960).

6. Symons, J.M. & Hoff, J.C., Rationale for Turbidity Maximum Contaminant Level. Proc., AWWA Water Quality Tech. Conf. (Dec. 1975).

7. Sanderson, W.W. & Kelly, S., Discussion of "Human Enteric Viruses in Water: Source, Survival and Removability" by Clark, N.A., Berg, G., Kabler, P.K. & Chang, S.L., Int. Conf. Water Pollut. and Res., presented London (1962). Pergamon Press (1964).

8. Hudson, H.E., High-Quality Water Production and Viral Disease, Jour. AWWA, 54:10 (1962).

9. Committee on Water Supply. Bacterial Aftergrowth in Water Distribution Systems. Am. Jour. Pub. Health, 20 (1930).

10. Bayliss, J.R., Bacterial Aftergrowth in Water Distribution Systems. Water Works and Sewerage, 77:10 (1930).

11. Howard, N.J., Bacterial Depreciation of Water Ouality in Distribution Systems, Jour. AWWA, 32:9 (1940).

12. Jewell, A.B., Bacterial Aftergrowth in the Distribution System. Southwest Water Works Jour., 23:13 (1942).

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
6-1/2"
3/8"

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

13. Hillmer, H.E., Data Concerning Study of Water Quality and its Relationship to New Water Distribution Systems in Private Homes Which Have Not Been Chlorinated. Presented to the City Managers and Common Council of the City of Fond du Lac, Wis. (Oct. 1970).

14. Lee, S.H. & O'Connor, J.T., Biologically Mediated Deterioration of Water Quality in Distribution Systems. Proc., AWWA Annu. Conf. (June 1975).

15. Victoreen, H.T., What's the Significance of Nuisance Organisms? Proc., AWWA Water Qual. Tech. Conf. (Dec. 1975).

16. Allen, M.J., Taylor, R.H. & Geldreich, E.E., The Impact of Excessive Bacterial Populations on Coliform Methodology. Proc., AWWA Water Qual. Tech. Conf. (Dec. 1976).

17. Geldreich, E.E., Taylor, R.H. & Allen, M.J., Bacterial Considerations in the Installation and Repair of Water Mains. Proc., AWWA Water Qual. Tech. Conf. (Dec. 1974).

18. Victoreen, H.T., Control of Water Quality in Transmission and Distribution Mains. Jour. AWWA, 66:6 (1974).

19. Allen, M.J. & Geldreich, E.E., Distribution Line Sediments and Bacterial Regrowths. Proc., AWWA Water Qual. Techn. Conf. (Dec. 1977).

20. Mackenthun, K.M. & Keup, L.E., Biological Problems Encountered in Water Supplies. Jour. AWWA, 62:8 (1970).

21. Shindala, A. & Chisholm, C.H., Water Quality Changes in the Distribution System. Water and Waste Eng., 7:1 (1970).

22. Victoreen, H.T., Soil Bacteria and Color Problems in Distribution Systems, Jour. AWWA, 61:9 (1969).

23. Geldreich, E.E., Nash, H.D. & Spino, D., Characterizing Bacterial Populations in Treated Water Supplies: A Progress Report. Proc., AWWA Water Qual. Tech. Conf. (Dec. 1977).

24. Hutchinson, M., WRA Medlube: An Aid to Main Disinfection. Water Treat. Exam, 23 (1974).

25. Buelow, R.W., et al, Disinfection of New Water Mains. Jour. AWWA, 68:6 (1976).

26. Victoreen, H.T., Water Quality Deterioration in Pipelines. Proc., AWWA Water Qual. Tech. Conf. (Dec. 1977).

27. Lee, S.H., O'Connor, J.T. & Banerji, S.K., Biologically Mediated Deterioration of Water Quality in Distribution Systems. Proc., AWWA Water Tech. Conf. (Dec. 1977).

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.) (4-76)

28. Ainsworth, R.G., Discoloured Water in Distribution Systems. Proc., AWWA Water Tech. Conf. (Dec. 1977).

29. Hawes, R.D., Disinfection of Reservoirs and Mains. Proc., AWWA Water Qual. Tech. Conf. (Dec. 1977).

30. Taylor, E.W., 42nd Rept. on the Results of the Bacteriological, Chemical and Biological Examination of the London Water for the Years 1965-1966. Metropolitan Board of Water, London.

31. Alter, A.J., Appearances of Intestinal Wastes in Surface Water Supplies at Ketchikan, Alaska. Proc. of 5th Alaska Sci. Conf. (Sept. 1954).

32. Geldreich, E.E., Is the Total Count Necessary? Proc., AWWA Water Qual. Tech. Conf. (Dec. 1973).

33. Robeck, G.G., Substitution of Residual Measurement for Distribution Bacteriological Sampling. Proc., AWWA Water Qual. Tech. Conf. (Dec. 1974).

34. McCabe, L.J., Symons, J.M., Lee, R.D. & Robeck, G.G., Community Water Supply Systems. Jour. AWWA, 62:11 (1970).

35. Buelow, R.W. & Walton, G., Bacteriological Quality as Residual Chlorine. Jour. AWWA, 63:1 (1971).

36. Knowlton, K.F., Knowlton, T.W., Forty Years - How They Went. Proc., AWWA Annu. Conf. (June 1976).

37. Warrington, R.A., Bacterial Contamination Problem Within the Water System, for the city of Salem, MA., C.E. Maguire, Inc., Boston, MA (1978).

38. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WPCF, Washington, D.C. (14th ed. 1976).

39. Laubusch, E., Chlorination and Other Disinfection Processes. (In Water Quality Treatment, AWWA). McGraw-Hill Book Co. (3rd ed. 1971).

40. Butterfield, C.T., Wattie, E., Megregin, S., Chambers, C.W., Influences of pH and Temperature on the Survival of Coliforms and Enteric Pathogens When Exposed to Free Chlorine. Public Health Rept., 58 (1943).

41. Butterfield, C.T., Bactericidal Properties of Free and Combined Chlorine. Jour. AWWA, 40 (1948).

42. Snow, W. & Brewster, W., Recommended Chlorine Residuals for Military Water Supplies. Jour. AWWA, 48 (1956).

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

43. Hill, M.T., et al. Manual of Instruction for Water Treatment Plant Operators. New York State Dept. Health, Albany, N.Y., (1970).

44. Baker, R., Carroll, L. & Laubusch, E., et al. Water Chlorination Principles and Practices. AWWA Man. of Water Supply Prac., M20, (1973).

45. Palin, A.T., Chemistry and Control of Modern Chlorination, LaMotte Chemical Products Co., Chestertown, MD (1973).

46. Safe Drinking Water Act, Public Law 93-523, Part B, Sec. 1412.

47. Crawford, H.B. & Fischel, D.N., ed., Water Quality and Treatment. McGraw-Hill Book Co, New York, NY (1971).

48. Bigger, J.W., Jour. of Pathol. and Bacteriol, 44 (1937).

49. Ptak, D.J., Ginsburg, W. & Willey, B.F., Identification and Incidences of KLEBSIELLA in Chlorinated Water Supplies. Jour. AWWA, 65:9 (1973).

50. Wellings, F.M. et al, Isolation of Enterovirus from Chlorinated Tap Water. Jour. Infect. Dis., in press.

9-1/8

51. Seidler, R.J., Morrow, J.E. & Bagley, S.T., KLEBSIELLA in Drinking Water Emanating from Redwood Tanks. Appl. Environ. Microbiol., 33 (1977).

52. Willis, A.T., Anaerobic Bacilli in Treated Water Supplies. Jour. Appl. Bacteriol., 20 (1957).

53. Leuchow, L.A. & Mackenthun, K.M., Detection and Enumeration of Iron Bacteria in Municipal Water Supplies. Jour. AWWA, 54 (1962).

54. Clark, F.M., Scott, R.M. & Bone, E., Heterotrophic Iron Precipitating Bacteria. Jour. AWWA, 7 (1970).

55. Ptak, D.J. & Ginsburg, W., Bacterial Indicators of Drinking Water Quality. Bacterial Indicators/Health Hazards Associated with Water, STP635 Hoadley, A.W. & Dutka, B.J., eds. ASTM, Phil. PA (1977).

56. Gates, W.H., The Halifax Story. Ontario Section AWWA. Seminar "Current Aspects of Potable Water Quality", Burlington, Ontario. (1979).

57. Fletcher, M. & Floodgate, G.D., An Electron Microscopic Demonstration of an Acidic Polysaccharide Involved in the Adhesion of Marine Bacterium to Solid Surface. Jour. Gen. Microbiol., 74 (1973).

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

58. Grant, W.D., Sutherland, I.W. & Wilkinson, J.F., Exopolysaccharide Colanic Acid and Its Occurrences in Enterobacteriaceae. Jour. Bacteriol., 100:2 (1969).

59. Moat, A.G., Microbial Physiology, John Wiley & Sons, N.Y. (1979).

60. Costerton, J.W., Geesey, G.G. & Cheng, K.J., How Bacteria Stick, Sci. Am., 238:1 (1978).

61. Costerton, J.W., Ingram, J.M. & Cheng, K.J., Structure and Function of the Cell Envelope of Gram Negative Bacteria. Bacteriol. Rev., 38:1 (1974).